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Onyx Dkt No. 1047.DIV
USSN: 10/669,768
PATENT

CERTIFICATE OF FIRST CLASS MAILING
I hereby certify that this correspondence is being deposited with the United States Postal Service under 37 CFR §1.8 on the date indicated below and with sufficient postage addressed to Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450.
Gary R. Fabian Printed Name
Gary R Fabian — 9 December 2008 Signature — Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE	
In Re Application of: SHEN, Y., et al.	Confirmation No.: 8135
Serial No.: 10/669,768	Art Unit: 1633
Filing Date: 24 September 2003	Examiner: Marvich, M.
Title: ADENOVIRUS E1B-55K SINGLE AMINO ACID MUTANTS AND METHODS OF USE	

TRANSMITTAL

Mail Stop: Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith for filing in the above-referenced application are the following documents:

1. Transmittal, including petition for extension of time and fee for Appeal Brief;
2. Brief on Appeal (including the Evidence Appendix, comprising Evidence Appendix A);
3. Certificates of First Class Mailing;
4. Return receipt postcard.

This is an appeal of the Office Action mailed on 15 January 2008, finally rejecting claims 11, 12, 24, 28, 33, 39 and 40. A Response and Amendment was filed by appellants on 15 July 2008. An Advisory Action was mailed on 14 August 2008. A Notice of Appeal in this application was filed on 15 July 2008. Accordingly, the Appeal Brief was due, without extension, on 15 September 2008. A

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Request for Three-Month Extension of Time accompanies this brief in the transmittal. Thus the due date of the Appeal Brief, with extension, is 15 December 2008.

Appellants request a Three-Month Extension of Time for filing of the accompanying brief under the provisions of 37 CFR 1.136(a) to extend the period for filing the brief in the above-identified application.

The requested extension and fee are as follows:

	Time Period	Fee	Total
	One month (37 CFR 1.17(a)(1))	130.00	
	Two months (37 CFR 1.17(a)(2))	490.00	
XX	Three months (37 CFR 1.17(a)(3))	1,110.00	$1,110.00
	Four months (37 CFR 1.17(a)(4))	1,730.00	
	Five months (37 CFR 1.17(a)(5))	2,350.00	

Authorization to Charge Deposit Account

The Commissioner is hereby authorized to charge to Deposit Account No. 15-0615 the required fee of **$1,110.00** for the three-month extension and the required fee of **$540.00** under 37 C.F.R. §41.20(b)(2) for filing a brief in support of an appeal (please reference **ONYX1047.DIV**). No additional fee is believed due; however, the Commissioner is hereby authorized to charge to Deposit Account No. 15-0615 any fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 which may be required by this paper, with the exception of the payment of the issue fee.

Respectfully submitted,

Dated: 9 Dec 2008

By: Gary R Fabian

Gary R. Fabian, Ph.D.
Registration No. 33,875
Agent for Appellants

ONYX Pharmaceuticals, Inc.
2100 Powell Street
Emeryville, CA 94608
Phone: (510) 597-6502
Facsimile: (510) 597-6610

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Gary R. Fabian
Printed Name

Gary R Fabian
Signature

9 December 2008
Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: SHEN, Y., et al.	Confirmation No.: 8135
Serial No.: 10/669,768	Art Unit: 1633
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Title: ADENOVIRUS E1B-55K SINGLE AMINO ACID MUTANTS AND METHODS OF USE	

BRIEF ON APPEAL

Mail Stop: Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is an appeal of the Office Action mailed on 15 January 2008, finally rejecting claims 11, 12, 24, 28, 33, 39 and 40. A Response and Amendment was filed by appellants on 15 July 2008. An Advisory Action was mailed on 14 August 2008. A Notice of Appeal in this application was filed on 15 July 2008. Accordingly, the Appeal Brief was due, without extension, on 15 September 2008. A Request for Three-Month Extension of Time accompanies this brief in the transmittal. Thus the due date of the Appeal Brief, with extension, is 15 December 2008. Authorization to charge the deposit account for the fee required under 37 CFR 41.20(b)(2) for filing an appeal brief accompanies this brief in the transmittal.

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PERSONAL APPEARANCE BEFORE THE BOARD OF APPEALS IS WAIVED

Appellants waive the opportunity for a personal appearance before the Board of Appeals to argue the issues of this appeal.

REAL PARTY IN INTEREST

The real party in interest in the present application is ONYX PHARMACEUTICALS, INC. The assignment of rights by the applicants of this application to Onyx Pharmaceuticals, Inc., is of record in the parent application to the present divisional. The parent application is now U.S. Patent No. 6,500,653 and the Reel/Frame numbers for the recorded assignment are as follows: 012602/0482 (recorded 02/11/2002).

RELATED APPEALS AND INTERFERENCES

Appellants are unaware of any prior or pending related appeals, interferences or judicial proceedings.

STATUS OF CLAIMS

Claims 11-13, 24-28, 33, and 35-40 are pending. Claims 11, 12, 24, 28, 33, 39 and 40 are rejected.

Claims 13, 25-27 and 35-38 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. See Office action, mailed 15 January 2008, page 8.

Claims 1-10, 14-23, 29-32, 34, and 41-47 are canceled.

The rejection of claims 11, 12, 24, 28, 33, 39 and 40 is appealed herein.

STATUS OF AMENDMENTS

The status of the amendment filed subsequent to final rejection is as follows: all amendments to the claims have been entered and all arguments and evidence presented in appellants' response to final rejection, mailed 15 July 2008 are of record for the purposes of appeal. *See* Advisory Action, mailed 14 August 2008, page 1.

SUMMARY OF THE CLAIMED SUBJECT MATTER

There are two groups of claims pending in the present application related to the two pending independent claims 11 and 33. Generally the claims are directed to methods of treating a cancer, characterized by neoplastic cells or a tumor comprising neoplastic cells that substantially lack p53 function, in a patient in need of the treatment.

In the first group of claims, corresponding to independent claim 11, the claims are directed to a method of treating a cancer, characterized by neoplastic cells that substantially lack p53 function, in a patient in need of the treatment (*see, e.g.,* specification page 1, lines 9-11; page 4, lines 1-9; page 6, line 25, to page 7, line 2; Abstract; and original claim 11). The method comprises (i) administering chemotherapy to the patient (*see, e.g.,* specification, page 4, lines 6-9; page 15, lines 27-30; and page 17, lines 6-19), (ii) administering to the patient a dose of a recombinant adenovirus (*see, e.g.,* specification, page 16, lines 26-32; and page 17, lines 3-5) the recombinant adenovirus comprising a mutation in the E1B-55K gene, the gene encoding a mutated E1B-55K protein comprising a single amino acid substitution mutation, the single amino acid substitution mutation reducing the ability of the mutated E1B-55K protein to bind to the tumor suppressor p53 when compared to the ability of wild-type E1B-55K protein to bind to the tumor suppressor p53 and the recombinant adenovirus has the further property of retaining late viral function (*see, e.g.,* specification, page 3, lines 31-34; page 4, lines 1-5; page 6, lines 11-13; and Example 3, page 22, line 8, to page 23, line 17), and (iii) allowing sufficient time for the recombinant adenovirus to infect neoplastic cells of the cancer (*see, e.g.,* specification, page 16, line 2, to page 17, line 19; and original claim 11).

The method may further comprise concomitantly administering the recombinant adenovirus with the chemotherapy (*see, e.g.,* pending claim 12). In some embodiments the adenovirus is Onyx 051 or Onyx 053 (*see, e.g.,* pending claims 13, 25, and 26). The method of treatment may be repeated (*see, e.g.,* pending claim 24). In some embodiments the mutated E1B-55K protein comprises a single amino acid substitution mutation in amino acid 240 or 260 (*see, e.g.,* pending claim 27). Further, in the method of treating cancer, replication of the recombinant adenovirus may be cold insensitive (*see, e.g.,* pending claim 28).

In the second group of claims, corresponding to independent claim 33, the claims are directed to a method of treating a cancer, characterized by a tumor comprising neoplastic cells that substantially lack p53 function, in a patient in need of the treatment (*see, e.g.,* specification page 1, lines 9-11; page 4, lines 1-9; page 6, line 25, to page 7, line 2; page 10, lines 4-13; Abstract; and original claim 11). The method comprises (i) administering chemotherapy to the patient (*see, e.g.,* specification, page 4, lines 6-9; page 15, lines 27-30; and page 17, lines 6-19), (ii) administering by direct injection into the tumor a dose of a recombinant adenovirus (*see, e.g.,* specification, page 15, lines 6-23), the recombinant adenovirus comprising a mutation in the E1B-55K gene, the gene encoding a mutated E1B-55K protein comprising a single amino acid substitution mutation, the single amino acid substitution mutation reducing the ability of the mutated E1B-55K protein to bind to the tumor suppressor p53 when compared to the ability of wild-type E1B-55K protein to bind to the tumor suppressor p53 and the recombinant adenovirus has the further property of retaining late viral function (*see, e.g.,* specification, page 3, lines 31-34; page 4, lines 1-5; page 6, lines 11-13; and Example 3, page 22, line 8, to page 23, line 17), and (iii) allowing sufficient time for the recombinant adenovirus to infect neoplastic cells of the cancer (*see, e.g.,* specification, page 16, line 2, to page 17, line 19; and original claim 11).

In some embodiments, the adenovirus is Onyx 051 or Onyx 053 (*see, e.g.,* pending claims 35, 36, and 37). In some embodiments the mutated E1B-55K protein comprises a single amino acid substitution mutation in amino acid 240 or 260 (*see, e.g.,* pending claim 38). Further, in the method of treating cancer, replication of the recombinant adenovirus may be cold insensitive (*see, e.g.,* pending claim 39). The method of treatment may be repeated (*see, e.g.,* pending claim 40).

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Sole Issue

In the final Office action, dated 15 January 2008, the Examiner rejected claims 11, 12, 24, 28, 33, 39 and 40 under 35 U.S.C. §112, first paragraph, asserting (i) that the specification, while being enabling for treatment of cancer characterized by p53 loss or deficiency by direct administration Onyx 051 and 053 (comprises a single amino acid substitution in amino acid 240 or 260), does not reasonably provide enablement for any other embodiment, and (ii) that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims. *See,* Office action, mailed 15 January 2008, page 2.

ARGUMENT

1.0.0 Sole Issue

In the final Office action, dated 15 January 2008, the Examiner rejected claims 11, 12, 24, 28, 33, 39 and 40 under 35 U.S.C. §112, first paragraph, asserting (i) that the specification, while being enabling for treatment of cancer characterized by p53 loss or deficiency by direct administration Onyx 051 and 053 (comprises a single amino acid substitution in amino acid 240 or 260), does not reasonably provide enablement for any other embodiment, and (ii) that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims. *See,* Office action, mailed 15 January 2008, page 2. This rejection is traversed for reasons discussed below.

1.1.0 Appellants Submit the Specification Provides Enablement Commensurate in Scope with the Claimed Subject Matter.

The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation. *See United States v. Telectronics, Inc.*, 857 F.2d 778, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988). *See, e.g., Ex Parte Forman*, 230 USPQ 546, 547 (P.T.O. Bd. Pat. App. & Int., 1986). A patent may be enabling even though some experimentation is necessary. *See United States v. Telectronics, Inc.*, 857 F.2d 778, 785, 8 USPQ2d 1217 (Fed. Cir. 1988).

In the present application, appellants have clearly enabled one of ordinary skill in the art to make and use the invention commensurate in scope with the claims without undue experimentation for the following reasons:

(i) <u>The Examiner has acknowledged that the efficacy of the present invention lies in the treatment of p53(-) tumors</u> (*see, e.g.,* specification, Example 4, page 23) and that the efficacy of <u>combined adenoviral/chemotherapy treatment has been specifically observed</u>, for example, as discussed by Kirn, et al. (*see* Office action, mailed 15 January 2008, paragraph bridging pages 3-4). Further, the Examiner has acknowledged the efficacy of the methods of the present invention by indicating allowable subject matter (*see* Office action, mailed 15 January 2008, page 8).

(ii) Appellants' specification explicitly <u>describes the methodology for the creation of the recombinant adenovirus of the present invention</u> as well as <u>methods of identifying recombinant adenoviruses having the desired characteristics</u> for use in the practice of the methods of the present invention.

(iii) Finally, the Examiner's asserted scope rejection regarding enablement of embodiments other than Onyx 051 and 053 is completely inconsistent with the fact that the <u>recombinant adenovirus recited in the independent claims of the present application mirrors the claim limitations in the independent claims that recited the same recombinant adenovirus of the **granted patent** of the parent application.</u>

The Examiner has not taken issue with enablement of any of the dependent claims and has focused arguments only on the elements of the independent claims. There are two pending independent claims -- claims 11 and 33. Claim 33 primarily differs from claim 11 in that claim 33 comprises "administering by direct injection into the tumor a dose of a recombinant adenovirus" and claim 11 comprises "administering to said patient a dose of a recombinant adenovirus." Accordingly, the following arguments, although focused on the limitations of claim 11, are equally applicable to the limitations of claim 33.

The Examiner's rejection focuses on the assertion that appellants' claims include "broad recitation of a genus of adenovirus for delivery to p53 lacking neoplastic cells wherein the adenovirus have reduced binding to p53" (*see* Office action, 15 January 2008, page 4; *see, also* Advisory Action, 14 August 2008, page 2, third paragraph).

The focus of the Examiner's rejection is further illustrated in the following remarks:

> However, by recitation that the rAd comprises a single amino acid mutation in EIB-55K, the adenovirus to be used in the treatment encompasses a broad and diverse genus of adenoviruses that need only be linked by a mutation in EIB-55K. Rather the nature of the adenoviruses for treatment of cancer according to the instant invention must be replicative. To this end, applicants generated 26 mutants but only two of these mutants are capable of reduced binding to p53. These mutants (Onyx 051 and 053) comprise a single mutation in amino acid 240 and 260.
>
> Hence, applicants have elucidated the unpredictability of any single amino acid to produce the required functional requirements as only two mutants of 26 produced have the recited functional requirements. As well, applicants have not provided the structural requirements of the single amino acid mutants such that one of skill in the art would be able to identify those mutants that have lost the ability to bind efficiently to p53. **Hence, the**

> **unpredictability of using the claimed invention in gene therapy is accentuated due to the broad and unpredictable nature of the identifying adenovirus with single amino acid mutations in the E1B 55k gene that have lost the ability to bind p53 and furthermore be used to treat cancer.** (*See* Office action, mailed 15 January 2008, pages 4-5; emphasis added.)

The Examiner has not presented any evidence to support the Examiner's assertion that "the unpredictability of using the claimed invention in gene therapy is accentuated due to the broad and unpredictable nature of the identifying adenovirus with single amino acid mutations in the E1B 55k gene that have lost the ability to bind p53 and furthermore be used to treat cancer." In the Advisory Action, mailed 14 August 2008, the Examiner repeats this assertion of unpredictablitiy (*see, e.g.,* Advisory Action, mailed 14 August 2008, page 2, fourth paragraph beginning "The MPEP teaches..."). However, these are no more than conclusionary statements unsupported by any evidence. Appellants, on the other hand, have presented extensive evidence that counters the Examiner's assertion of "unpredictability."

First, <u>the Examiner has acknowledged that the efficacy of the present invention lies in the treatment of p53(-) tumors</u> and that the efficacy of <u>combined adenoviral/chemotherapy treatment have been specifically observed</u>, for example, as discussed by Kirn, et al. (*see* Office action, mailed 15 January 2008, paragraph bridging pages 3-4). Further, the Examiner has acknowledged the efficacy of the claimed methods of treating cancer by indicating allowable subject matter (*see* Office action, mailed 15 January 2008, page 8). The following arguments by appellants were previously presented in appellants' Response to Final Rejection, mailed 15 July 2008, pages 4-6.

The reference of Kirn, et al., is of record in the present application and a copy is presented in Evidence Appendix A of this Brief. Kirn, et al., discuss the use of adenovirus mutant *dl*1520 (ONYX-015) in clinical trials for the treatment of a number of cancer types. As discussed by Kirn, et al.:

> *dl*1520 (Onyx-015) was the first adenovirus described to mirror the gene deletion approach pioneered by Martuza with herpesvirus. Bischoff *et al.* (1996) hypothesized that an adenovirus with deletion of a gene encoding a p53-binding protein, E1B-55 kD, would be selective for tumors that already had inhibited or lost p53 function. p53 function is lost in the majority of human cancers through mechanisms including gene mutation, overexpression of p53-binding inhibitors (e.g., mdm2, human papillomavirus E6) and loss of the p53-inhibitory pathway modulated by p14^{arf}. (*See* Kirn, et al., page 6653, col. 1.)

Kirn, et al., provide extensive discussion of the use of adenovirus ONYX-015 alone and in combination with chemotherapy for the treatment of cancer. Kirn, et al., state the following with regard to ONYX-015:

> For the first time since viruses were first conceived as agents to treat cancer over a century ago, we now have definitive data from numerous phase I and phase II clinical trials with a well-characterized and well-quantitated virus. *dl*1520 (Onyx-015, now CI-1042, Pfizer, Inc.) is a novel agent with a novel mechanism of action. This virus was to become the first virus to be used in humans that had been genetically-engineered from replication-selectivity. (Kirn, et al., page 6664, col. 2).

Further, Kirn, et al., discuss the evidence for a potential-synergistic interaction between adenoviral therapy and chemotherapy that has been demonstrated in multiple clinical trials (Kirn, et al., page 6666, col. 1, to 6667, col. 1).

Kirn, et al., conclude:

> Replication-selective oncolytic adenovirus represent a novel cancer treatment platform. Clinical studies have demonstrated the safety and feasibility of the approach, including the delivery of adenovirus to tumors through the bloodstream (Heise *et al.*, 1999b; Reid *et al.*, 1999; Nemunaitis *et al.*, 1999). The inherent ability of replication-competent adenoviruses to sensitize tumor cells to chemotherapy was a novel discovery that has led to chemosensitization strategies. (Kirn, et al., page 6667, col. 2).

In the present application, <u>appellants have consistently compared important phenotypes of ONYX-015 to the replication-selective recombinant adenoviruses of the present invention</u>. Appellants demonstrated that the recombinant adenoviruses of the present invention (i) showed substantially reduced binding of p53 (as does ONYX-015; *see* specification, Example 2, pages 19-22), (ii) showed protein synthesis profiles more similar to wild-type than to ONYX-015 which is an <u>advantage</u> because generally higher levels of adenoviral replication correspond to increased cytotoxicity in target cells (*see* specification, Example 3, pages 22-23), and, consistent with the previous observation, (iii) tumor cell specific cytolytic activity of the recombinant viruses of the present invention was <u>higher</u> than ONYX-015 (*see* specification, Example 4, page 23). Thus, it is clear from the data presented by appellants that the recombinant viruses of the present invention provide at least

similar if not superior anti-cancer properties relative to ONYX-015, which has been demonstrated in clinical trials to be useful for the treatment of cancers.

One important previously unrecognized advantage of recombinant adenovirus of the present invention, comprising the single amino acid mutations, resides in the observation that recombinant adenoviruses comprising a single amino acid substitution mutation in the E1B-55K gene resulted in recombinant adenoviruses that had replication capacity in human cancer cells more like wild-type adenovirus versus the attenuated replication capacity of ONYX-015 (*see, e.g.*, specification page 12, lines 10-20). This increased ability to replicate in human cancer cells results in improved tumor cytolytic activity relative to, for example, ONYX-015 (*see, e.g.*, specification, page 23, lines 20-35).

Further, the present specification discusses the combination of the claimed recombinant adenoviruses with chemotherapy (see, e.g., specification, pages 16-17).

The Examiner has presented no evidence to support the assertion that use of the recombinant adenovirus of the present invention in a method of treating a cancer, characterized by neoplastic cells that substantially lack p53 function, is "unpredictable." As discussed above, the Examiner's assertions are contradicted by the teachings of the specification and the teachings of Kirn, et al.

Second, the Examiner asserts "applicants have elucidated the unpredictability of any single amino acid to produce the required functional requirements as only two mutants of 26 produced have the recited functional requirements" (*see* Office action, mailed 15 January 2008, page 5). The following arguments by appellants were previously presented in appellants' Response to Final Rejection, mailed 15 July 2008, pages 7-9.

Contrary to the Examiner's assertion, the specification contains extensive teachings regarding making of the recombinant adenoviruses used in the methods of the present invention as illustrated by the following. Adenovirus E1B-55K protein sequences and nucleic acid coding sequences are well known in the art (*see, e.g.*, specification, pages 1-3; page 6, lines 7-10; page 9, lines 24-32; page 10, line 31 to page 11, line 19; page 12, lines 7-9; Example 1, pages 17-19). Specifically, the region of the E1B-55K protein that mediates its interaction with the p53 protein has been mapped (*see, e.g.*, specification, page 10, lines 31-34). Methods of constructing adenoviral mutants are known in the art (*see, e.g.*, specification, page 11, lines 15-28; page 12, lines 3-9). Guidance concerning substitution of

amino acids suitable for generating mutant polypeptides is discussed in the specification (*see, e.g.*, specification, page 12, line 21, to page 13, line 23). Tumor cell lines used to conduct screening of recombinant adenovirus are readily available (*see, e.g.*, specification page 11, line 29, to page 12, line 2). Thus, the specification clearly teaches one of ordinary skill in the art how to make and use the recombinant adenovirus of the present invention.

Further, one of ordinary skill in the art can use standard methods of molecular biology (*see, e.g.,* specification, page 5, line 26, to page 6, line 4) in view of the teachings of the present specification to generate the recombinant adenovirus claimed in the methods of the present invention. A patent need not teach, and preferably omits, what is well known in the art. *See Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.,* 730 F.2d 1452, 1453, 221 USPQ 481, 489 (Fed. Cir. 1984). *See Hybritech Inc. v. Monoclonal Antibodies*, 802 F.2d at 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986).

In addition, <u>appellants have provided a disclosure that explicitly describes the methodology</u> for the creation of the recombinant adenovirus of the present invention as well as methods of identifying recombinant adenoviruses having the desired characteristics for use in the practice of the methods of the present invention. Example 1 (*see* specification, pages 17-19) describes construction <u>of twenty-five single amino acid substitution E1B-55K mutant adenoviruses and one mutant having two amino acid substitutions.</u> Each recombinant adenovirus was generated using a forward primer and a reverse primer. Final products were transformed into XL-1 cells and confirmed by DNA sequencing. Recombinant viruses were constructed by co-transfecting pJM17 with plasmids containing the mutations into 293 cells and were plaque purified to rule out wild-type contamination. Mutations were confirmed by PCR followed by sequencing of the E1B-55K region.

Example 2 (*see* specification, pages 19-22) describes evaluation of binding of the isolated E1B-55K mutants with p53. The twenty six mutant adenoviruses produced as described in Example 1 were initially screened to determine their effect on the steady-state levels of p53 in A549 cells. These data suggested that two of the adenoviral E1B-55K mutants, R240A and H260A fail to bind p53. This was confirmed by directly examining the ability of the E1B-55K mutants R240A and H260A to interact with p53 by immunoprecipitation experiments using S^{35}-labeled cell extracts from infected A549 cells.

Example 3 (*see* specification, pages 22-23) describes the effects of the E1B-55K mutations on the ability of the recombinant adenoviruses to replicate in target cells. The ability to replicate in target tumor cells generally improves the cytotoxicity of the recombinant adenovirus. At 39°C., all of the viruses replicated to similar extent. The yield of *dl*309 was approximately 4-fold higher than that of ONYX-015, and the yields of ONYX-051 (mutant R240A) and ONYX-053 (H260A) fell in between. At 32°C., however, the ONYX-015 yield was reduced nearly 35-fold compared to that of *dl*309, which is consistent with the previous reports. Replication of ONYX-051 was essentially identical to that of *dl*309, while replication of ONYX-053 was slightly reduced (4-fold). Thus, ONYX-051 (mutant R240A) and ONYX-053 (H260A) have an improved ability to replicate in target cells relative to ONYX-015. Further, the protein synthesis profile in cells infected with ONYX-051 (mutant R240A) and ONYX-053 (H260A) was similar to that in cells infected with wild-type viruses *dl*309 and WtD. This observation suggests that ONYX-051 (mutant R240A) and ONYX-053 (H260A) are capable of modulating mRNA trafficking in favor of late viral mRNA nuclear export.

Example 4 (*see* specification, page 23) describes the cytotoxic activity of recombinant adenoviral E1B-55K mutants. Among the recombinant adenoviruses described in the application, most, including ONYX-051, were comparable to *dl*309 in their ability to infect and kill tumor cells. In the case of ONYX-053, its tumor cytolytic activity was 35- to 100-fold lower than that of *dl*309, <u>but more active than</u> <u>ONYX-015</u> by a factor of 4- to 5-fold.

Accordingly, two recombinant adenoviruses were identified out of twenty-six recombinant adenoviruses screened, using the methods described in the present application, that met the criteria for use in a method of treating cancer as outlined above. Further, important properties of the recombinant adenoviruses of the present invention were evaluated relative to the properties of ONYX-015, which the Kirn, et al., reference discusses in the context of a novel treatment platform using replication-selective adenoviruses and clinical trials for cancer treatment that used ONYX-015.

The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation *See Ex parte Forman*, 230 USPQ 546 (P.T.O. Bd. Pat. App. & Int., 1986). The law does not require the impossible. Hence, it does not require that an

applicant describe in his specification every **conceivable** and possible future embodiment of his invention. *See SRI International v. Matsushita Elec. Corp. of America*, 775 F.2d 1107, 1121, 227 USPQ 577 (Fed. Cir. 1985); emphasis in original. Further, the enablement requirement may be satisfied even though some experimentation is required. *See Hybritech Inc. v. Monoclonal Antibodies*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986).

In the Advisory Action, mailed 14 August 2008, the Examiner asserts the following:

> Even should applicants limit the claims to consisting of this single amino acid substitution, there are still 26 mutants that by not functioning indicate that the question is not undue experimentation as it appears that no amount of experimentation will identify other single amino acid substitutions that have reduced p53 binding and treat cancer in combination with chemotherapy. (*See* Advisory Action, page 2, last sentence of third paragraph.)

This assertion by the Examiner is conclusionary and unsupported by any evidence. The Examiner has no basis to draw the conclusion that "no amount of experimentation will identify other single amino acid substitutions." As discussed herein above, appellants have provided extensive guidance to those of ordinary skill in the art concerning the isolation and characterization of recombinant adenovirus useful in the practice of the present invention.

Accordingly, the specification describes in detail how to make recombinant adenoviruses comprising a single amino acid substitution mutation in the E1B-55K gene, how to determine that the mutation reduces the ability of the mutated E1B-55K protein to bind to the tumor suppressor p53, how to determine the recombinant adenoviruses that retain late viral function, and how to determine killing of neoplastic cells that substantially lack p53 function.

Appellants have taught one reasonably skilled in the art to make and use the invention from the disclosures in the application coupled, if necessary, with information known in the art without undue experimentation. Appellants described two specific embodiments of the recombinant adenoviruses of the present invention. It is it not required that appellants describe in the specification every **conceivable** and possible future embodiment of the invention.

Finally, appellants respectfully point out that application USSN 09/918,696, now U.S. Patent No. 6,635,244, is the parent application to the present application, <u>was examined by the same Examiner,</u> and comprises the following <u>granted</u> claims:

1. A recombinant adenovirus comprising a mutation in the E1B-55K gene, said gene encoding a mutated E1B-55K protein comprising a single amino acid mutation, said single amino acid mutation reducing the ability of said E1B-55K mutated protein to bind to the tumor suppressor p53 when compared to the wild-type E1B-55K protein and said adenovirus has the further property of retaining late viral function.

2. A recombinant adenovirus as described in claim 1, wherein said adenovirus is selected from the group consisting of Onyx 051 and Onyx 053.

3. A recombinant adenovirus as described in claim 2 wherein said adenovirus is Onyx 051.

4. A recombinant adenovirus as described in claim 2 wherein said adenovirus is Onyx 053.

5. A recombinant adenovirus as described in claim 1, wherein said adenovirus has a mutation in amino acid 240 or 260.

6. A recombinant adenovirus as described in claim 1, wherein the replication of said adenovirus is cold insensitive.

As <u>the parent application is now an issued U.S. Patent</u>, the presumption of validity under 35 U.S.C. §282 carries with it the presumption that the Examiner did the Examiner's duty and knew what claims the Examiner was allowing. The <u>recombinant adenovirus in the independent claims of the present application mirror the claim limitations in the independent claims of the granted patent of the parent application.</u> Accordingly, appellants submit that it is completely inappropriate for the Examiner to be questioning the scope of the presently claimed invention based on a question of whether or not one of ordinary skill in the art is capable of making or using the claimed recombinant adenovirus in a method of treating cancer. The claims of the granted parent application support that making of the required recombinant adenoviruses is enabled by the teachings of the present specification. The teachings of the present application, as further evidenced by the teachings of the reference of Kirn, et al., enable use of the claimed recombinant adenovirus in the methods of the present invention. These arguments by appellants were previously presented in appellants' Response to Final Rejection, mailed 15 July 2008, pages 9-11.

In the present application, appellants have isolated and characterized recombinant adenovirus comprising a single amino acid substitution mutation in the E1B-55K gene that

reduces the ability of the mutated E1B-55K protein to bind to the tumor suppressor p53, wherein the recombinant adenovirus retains late viral function. Appellants were the first to demonstrate that single amino acid mutations were capable of reducing or eliminating E1B-55K protein's ability to bind to the tumor suppressor p53 and that recombinant adenoviruses harboring such single amino acid mutations demonstrated tumor cytotoxicity.

In the Advisory Action, mailed 14 August 2008, the Examiner asserts the following:

> The MPEP teaches that the [*sic*] "As to the patentability of the instant case in light of similar claims in published patents that possess similar disclosures, rejections based upon this argument have been addressed in in [*sic*] re Giolito and Hoffman." It is immaterial whether similar claims have been allowed to others" (see in [*sic*] re Giolito and Hoffman 188 USPQ 645). Rather, each application is reviewed on its own merits." In this case, applicants have not described additional single amino acid mutations. *See* Advisory Action, mailed 14 August 2008, page 2, fourth paragraph beginning "The MPEP teaches...."

Appellants were unable to confirm the Examiner's citation from the MPEP referencing *In re Giolito and Hoffman*; however, in *In re Giolito and Hofmann*, 530 F.2d 397, 188 USPQ 645, 648 (Fed. Cir. 1976) the Federal Circuit stated the following:

> We reject appellants' argument that the instant claims are allowable because similar claims have been allowed in a patent. It is immaterial whether similar claims have been allowed to others. See *In re Margaroli*, 50 CCPA 1400, 318 F.2d 348, 138 USPQ 158 (1963); *In re Wright*, 45 CCPA 1005, 256 F.2d 583, 118 USPQ 287 (1958); *In re Launder*, 41 CCPA 887, 212 F.2d 603, 101 USPQ 391 (1954).

Further, the issue addressed by the Federal Circuit in *In re Giolito and Hofmann* was a rejection under 35 U.S.C. §103. Appellants submit that the Examiner's reliance on *In re Giolito and Hoffman* is improper because in the present application the granted patent that is referred to is <u>the parent patent</u> of the present application and <u>not</u> "similar claims allowed to <u>others</u>" (emphasis added). Further, the Examiner of the present application also examined the parent patent. Finally, the rejection being asserted by the Examiner in the present application is a rejection under 35 U.S.C 112, first paragraph, <u>not</u> a rejection under 35 U.S.C. §103 as was being considered in *In re Giolito and Hofmann.*

In view of the above arguments, appellants submits that the claims are enabled for the entire scope of the claimed invention. Appellants respectfully request that the Examiner's

rejection of the claims under 35 U.S.C 112, first paragraph, be reversed.

1.2.0. The Examiner Has Failed to Establish a *Prima Facie* Case of Lack of Enablement Commensurate in Scope with the Claimed Subject Matter.

To support the rejection of the claims for lack of enablement commensurate in scope with the claimed subject matter, the Examiner stated the following:

> The MPEP teaches, "However, claims reading on significant numbers of inoperative embodiments would render claims non-enabled when the specification does not clearly identify the operative embodiments and undue experimentation is involved in determining those that are operative. Atlas Powder Co. v. E.I. duPont de Nemours & Co., 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984); In re Cook, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971). (see MPEP 2164.08(b). In the instant case, applicants recite use of a broad genus of adenovirus to treat cancer. The instant rejection is based upon the highly unpredictable nature of the claimed method of treatment of *any* cancer using *any* of a broad genus of adenovirus. The lack of guidance as to the molecules to be used exacerbates the highly unpredictable nature of treating cancer. While one of skill in the art can readily envision numerable species of nucleic acid sequences that have at least a single amino acid mutation in EIB 55k, one cannot predict which of these also generate an adenovirus that treats cancer. (*See* Office action, mailed 15 January 2008, page 6.)

The following arguments by appellants were previously presented in appellants' Response to Final Rejection, mailed 15 July 2008, pages 11-17. Although the Examiner cites *Atlas Powder* and *In re Cook*, the Examiner has done nothing more than assert that the specification "does not clearly identify operative embodiments and undue experimentation is involved in determining those that are operative." On the other hand, as discussed herein above, the specification clearly teaches how to make and use the recombinant adenovirus of the present invention. Further, when confronted with a similar pattern of facts in the field of recombinant, molecular biology the Board of Patent Appeals and Interferences (B.P.A.I.) found that the Examiner had not established a reasonable basis for questioning the sufficiency of the supporting disclosure when taken in combination with the relevant state of the art as it related to the claimed invention. In *Ex parte Chen*, 61 USPQ2d 1025 (B.P.A.I. Aug. 22, 2001) the invention as claimed was to a transgenic carp containing an exogenous

gene encoding a growth hormone (the rtGH gene) that was operably linked to a promoter. This exogenous rtGH gene was introduced into the carp at an embryonic stage.

Certain claims were rejected by the Examiner who asserted the specification did not disclose a process that was repeatable as to the levels of expression to obtain carp or other fish that expressed the same transgene product, wherein the level of expression was shown to directly affect phenotypic characteristics of the fish. In support of the rejection, the Examiner cited a prior art reference as evidence of a level of unpredictability in this art. The reference taught that there are three steps or factors that must be shown to exist in a true transgenic animal: (1) integration into the host chromosome, (2) expression, and (3) germ-line transmission of foreign genes.

The applicant (Chen) did not dispute the three factor test presented by the Examiner but argued that the specification would meet this test and permit a person skilled in this art to make and use the claimed invention by following the detailed procedures disclosed in applicant's specification. *See Ex parte Chen*, 61 USPQ2d 1025, 1028 (B.P.A.I. Aug. 22, 2001).

Regarding the overall inquiry concerning enablement the B.P.A.I. stated the following:

> We are mindful that the Patent and Trademark Office (PTO) bears the initial burden of providing reasons for doubting the objective truth of the statements made by appellants as to the scope of enablement. Only when the PTO meets this burden, does the burden shift to appellants to provide suitable evidence indicating that the specification is enabling in a manner commensurate in scope with the protection sought by the claims. *See Ex parte Chen*, 61 USPQ2d 1025, 1027 (B.P.A.I. Aug. 22, 2001) (unpublished).

The B.P.A.I. reversed the rejections asserted by the Examiner and held:

> In responding to [Chen's] arguments, the examiner urges that the level of experimentation is undue and points to the success rate 1% or 20 out of 1746 attempts for the integration of the gene into the embryos described in the specification. However, **the examiner offers no evidence which would reasonably support a conclusion that one skilled in this art would regard this rate of success for the integration of the rtGH gene as evidencing undue experimentation.** We remind the examiner that some experimentation may be required as long as it is not undue .... [Chen's] **disclosure explicitly describes the methodology** to be used to arrive at the claimed transgenic carp. **As the record now stands, the numbers emphasized by the examiner**

> **would reasonably appear to reflect the need for a repetitive procedure, rather than undue experimentation by those wishing to practice the invention.** (*See Ex parte Chen*, 61 USPQ2d 1025, 1028 (B.P.A.I. Aug. 22, 2001) (unpublished); emphasis added.)

The B.P.A.I. found that the Examiner had not established a reasonable basis for questioning the sufficiency of the supporting disclosure when taken in combination with the relevant state of the art as it related to the claimed invention. The B.P.A.I. reversed the scope rejection under 35 U.S.C. §112, first paragraph.

In the present case, as in *Ex parte Chen*, the Examiner has provided no evidence that would reasonably support the conclusion that one skilled in the art would regard the success rate of 2/26 (~7.7%) of obtaining recombinant adenovirus (having the desired characteristics for use in the practice of the methods of present invention) as evidence of undue experimentation. Further, the Examiner presented no evidence to support the assertion that "it appears that no amount of experimentation will identify other single amino acid substitutions that have reduced p53 binding and treat cancer in combination with chemotherapy" (*see* Advisory Action, page 2, last sentence of third paragraph). Particularly in view of the fact that appellants have provided a disclosure that explicitly describes the methodology to be used to arrive at the claimed recombinant adenovirus having the desired characteristics for use in the practice of the methods of present invention. The numbers emphasized by the Examiner appear to do no more than reflect that the specification provides a repeatable procedure, rather than undue experimentation, for use by those having ordinary skill in the art to practice the present invention.

Whenever the PTO makes a rejection for failure to teach and/or use the invention, the PTO must explain its reasons for the rejection and support the rejection with (i) acceptable evidence, or (ii) reasoning which contradicts the applicants' claim: the reasoning must be supported by current literature as a whole and the PTO must prove the disclosure requires undue experimentation. *See In re Marzocchi*, 439 F.2d 220, 223-24, 169 USPQ 367, 369-70 (C.C.P.A. 1971). The Examiner has presented no evidence to support the Examiner's questioning of the objective enablement of the claims by the specification. The discussion presented by the Examiner amounts only to conclusions not supported by any evidence or reasoning supported by the current literature.

On the contrary, appellants provided extensive teachings relating to the generation and identification of recombinant adenovirus comprising a single amino acid substitution mutation in the E1B-55K gene that reduces the ability of the mutated E1B-55K protein to bind to the tumor suppressor p53, wherein the recombinant adenovirus retains late viral function. These teachings were discussed in detail herein above. Further, the teachings of the present specification regarding the properties of the claimed recombinant adenoviruses (for example, reduced binding to p53, retention of late viral function and replication in target cells, and tumor cell cytotoxicity) completely support the method claims of the present invention. As discussed above, the reference of Kirn, et al., also supports, by comparison to results of clinical trials of cancer treatments employing ONYX-015, use of the recombinant adenovirus of the present invention in methods of cancer treatment.

The Examiner states that "applicants have elucidated the unpredictability of any single amino acid to produce the required functional requirements as only two mutants of 26 produced have the recited functional requirements" (*see* Office action, mailed 15 January 2008, page 5). Once again, this statement is merely a conclusion without any evidence to support it. The Examiner has NOT provided any evidence to support a conclusion that finding 2/26 mutants (or 7.7% of recombinant viruses screened) with the desired characteristics constitutes undue experimentation. The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation. *See Ex parte Forman*, 230 USPQ 546 (P.T.O. Bd. Pat. App. & Int., 1986). Further, the enablement requirement may be satisfied even though some experimentation is required. *See Hybritech Inc. v. Monoclonal Antibodies*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986).

The Examiner also asserts "[t]he instant rejection is based upon the highly unpredictable nature of the claimed method of treatment of *any* cancer using *any* of a broad genus of adenovirus" (*see* Office action, mailed 15 January 2008, page 6; emphasis in original). This statement mischaracterizes the claims as the claims (i) are not directed to the treatment of "*any* cancer", rather the claims are directed to a method of treating a cancer, characterized by neoplastic cells that substantially lack p53 function, and (ii) are not directed to "*any* of a broad genus of adenovirus," rather the claims are directed to a "recombinant adenovirus comprising a mutation in the E1B-55K gene, said gene encoding a mutated E1B-

55K protein comprising a single amino acid substitution mutation, said single amino acid substitution mutation reducing the ability of said mutated E1B-55K protein to bind to the tumor suppressor p53 when compared to the ability of wild-type E1B-55K protein to bind to the tumor suppressor p53 and said recombinant adenovirus has the further property of retaining late viral function."

The Examiner goes on to assert that "[t]he lack of guidance as to the molecules to be used exacerbates the highly unpredictable nature of treating cancer" (*see* Office action, mailed 15 January 2008, page 6). Once again, however, this is an assertion unsubstantiated by any evidence. Contrary to this assertion the specification provides a great deal of guidance concerning how to make and use the recombinant adenovirus, having the claimed characteristics, in methods of treating cancer, as discussed herein above.

Further, the Examiner asserts "[w]hile one of skill in the art can readily envision numerable species of nucleic acid sequences that have at least a single amino acid mutation in EIB 55k, one cannot predict which of these also generate an adenovirus that treats cancer" (*see* Office action, mailed 15 January 2008, page 6). Once again, however, this is an assertion unsubstantiated by any evidence. Contrary to this assertion the specification provides guidance concerning how to generate the recombinant adenovirus of the present invention (*see, e.g.,* specification, Example 1, pages 17-19), how to identify recombinant adenovirus having a mutated E1B-55K protein with reduced ability to bind to the tumor suppressor p53 when compared to the ability of wild-type E1B-55K protein to bind to the tumor suppressor p53 (*see, e.g.,* specification, Example 2, pages 19-22), how to identify recombinant adenovirus with the property of retaining late viral function (*see, e.g.,* specification, Example 3, pages 22-23), and <u>how to identify recombinant adenovirus with increased killing of neoplastic cells that substantially lack p53 function</u> (*see, e.g.,* specification, Example 4, page 23).

In the Office action, mailed 15 January 2008, the Examiner invokes the holdings of *Amgen Inc.*, *Fiers*, and *Reagents of the Univ. of Calif.* to assert the following:

> an <u>adequate written description of a nucleic acid</u> requires more than a mere statement that it is part of the invention and reference to a potential method for isolating it, irrespective of the complexity or simplicity of the method; <u>what is required is a description of the nucleic acid itself</u>. It is not sufficient to define DNA solely by its principal biological property, because disclosure of no

> more than that, as in the instant case, is simply a wish to know the identity of any DNA with that biological property. Naming a type of material generically known to exist, in the absence of knowledge as to what that material consists of, is not a description of that material. When one is unable to envision the detailed constitution of a complex chemical compound having a particular function, such as a nucleic acid, so as to distinguish it from other materials, as well as a method for obtaining it, conception has not been achieved until reduction to practice has occurred, i.e., until after the nucleic acid has been isolated. Thus, claiming all DNA's that achieve a result without defining what means will do so is not in compliance with the description requirement. (*See* Office action, mailed 15 January 2008, pages 6-7; emphasis added.)

First, appellants point out that the present rejection is a scope of enablement rejection under 35 U.S.C. ¶112, first paragraph, NOT a written description rejection. Second, appellants point out that the claims are drawn to recombinant adenovirus having defined properties NOT to an isolated nucleic acid sequence *per se*. Third, regarding "chemical compounds," appellants remind the Examiner of the holding in *In re Papesch* that stated:

> From the standpoint of patent law, a compound and all of its properties are inseparable; they are one and the same thing. The graphic formulae, and the chemical nomenclature, the systems of classification and study such as the concepts of homology, isomerism, etc., are mere symbols by which compounds can be identified, classified, and compared. But a formula is not a compound and while it may serve in a claim to identify what is being patented, as the metes and bounds of a deed identify a plot of land, the thing that is patented is not the formula but the compound identified by it. *See The Regents of the University of New Mexico v. Knight and Scallen,* 321 F.3d 1111, 1122, 66 U.S.P.Q.2D 1001 (Fed. Cir. 2003) *citing In re Papesch,* 315 F.2d 381, 391, 137 U.S.P.Q. 43, 51 (CCPA 1962).

How to make and use the recombinant adenovirus having the claimed properties of the present invention is extensively described by the specification as described herein above. Accordingly the Examiner's reliance on the holdings of *Amgen Inc.*, *Fiers*, and *Reagents of the Univ. of Calif.* is improper and does not bear on the claims or the asserted rejection at issue.

Finally, the Examiner asserts the following:

> As to applicants arguments that "it is inappropriate for the Examiner to be questioning the scope of the presently claimed invention based on a question of whether or not one of the ordinary skill in the art is capable of "identifying adenovirus with single amino acid mutations in the El B 55k gene that have lost the ability to bind to p53", this statement omits the central issue

> at question which is in the remainder of the rejection "<u>and furthermore be used to treat cancer</u>". (*See* Office action, mailed 15 January 2008, page 7; emphasis added.)

Appellants provided extensive teachings relating to the generation and identification of recombinant adenovirus comprising a single amino acid substitution mutation in the E1B-55K gene that reduces the ability of the mutated E1B-55K protein to bind to the tumor suppressor p53, wherein the recombinant adenovirus retains late viral function. The details of these teachings were discussed herein above. Regarding the use of the recombinant adenovirus in methods of treating cancer, the teachings of the present specification concerning the properties of the claimed recombinant adenoviruses (for example, reduced binding to p53, retention of late viral function and replication in target cells, and tumor cell cytotoxicity) completely support the method claims of the present invention. As discussed above, the reference of Kirn, et al., teaches the use of ONYX-015 in clinical trials for viral therapy of cancer, in particular discussing a potential-synergistic interaction between adenoviral therapy and chemotherapy that has been demonstrated in multiple clinical trials (Kirn, et al., page 6666, col. 1, to 6667, col. 1). In the present application, <u>appellants have consistently compared important phenotypes of ONYX-015 to the replication-selective recombinant adenoviruses of the present invention</u>.

Appellants demonstrated that the recombinant adenoviruses of the present invention (i) showed substantially reduced binding of p53 (as does ONYX-015; *see* specification, Example 2, pages 19-22), (ii) showed protein synthesis profiles more similar to wild-type than to ONYX-015 which is an advantage because generally higher levels of adenoviral replication correspond to increased cytotoxicity in target cells (*see* specification, Example 3, pages 22-23), and, consistent with the previous observation, (iii) tumor cell specific cytolytic activity of the recombinant viruses of the present invention was higher than ONYX-015 (*see* specification, Example 4, page 23). Thus, it is clear from the data presented by appellants that the recombinant viruses of the present invention provide at least similar if not superior anti-cancer properties relative to ONYX-015, which has been demonstrated <u>in clinical trials</u> to be useful for the treatment of cancers.

Accordingly, in view of the above arguments, appellants submit that <u>the Examiner has failed to establish a *prima facie* case for lack of enablement of the present invention due</u>

to undue experimentation.

1.2.0 Conclusion

Appellants respectfully submit that the rejection of claims 11, 12, 24, 28, 33, 39 and 40 under 35 U.S.C. §112, first paragraph, which asserted (i) that the specification, while being enabling for treatment of cancer characterized by p53 loss or deficiency by direct administration Onyx 051 and 053 (comprises a single amino acid substitution in amino acid 240 or 260), does not reasonably provide enablement for any other embodiment, and (ii) that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims, should be reversed.

Conclusion

For the foregoing reasons, appellants respectfully submit that the Examiner has erred in rejecting claims 11, 12, 24, 28, 33, 39 and 40 of this application. Please reverse the Examiner on all counts.

Respectfully submitted,

Date: 9 Dec 2008

By: Gary R Fabian

Gary R. Fabian, Ph.D.
Registration No. 33,875
Agent for Appellants

ONYX Pharmaceuticals, Inc.
2100 Powell Street
Emeryville, CA 94608
Phone: (510) 597-6502
Facsimile: (510) 597-6610.

CLAIMS APPENDIX

1-10. (Canceled)

11. (Rejected) A method of treating a cancer, characterized by neoplastic cells that substantially lack p53 function, in a patient in need of the treatment, comprising

administering chemotherapy to said patient,

administering to said patient a dose of a recombinant adenovirus, said recombinant adenovirus comprising a mutation in the E1B-55K gene, said gene encoding a mutated E1B-55K protein comprising a single amino acid substitution mutation, said single amino acid substitution mutation reducing the ability of said mutated E1B-55K protein to bind to the tumor suppressor p53 when compared to the ability of wild-type E1B-55K protein to bind to the tumor suppressor p53 and said recombinant adenovirus has the further property of retaining late viral function, and

allowing sufficient time for said recombinant adenovirus to infect neoplastic cells of said cancer.

12. (Rejected) The method of claim 11, further comprising concomitantly administering said recombinant adenovirus with the chemotherapy.

13. (Objected To) The method of claim 11, wherein said adenovirus is Onyx 051 or Onyx 053.

14-23. (Canceled)

24. (Rejected) The method of treating cancer of claim 11, wherein said treatment is repeated.

25. (Objected To) The method of treating cancer of claim 13, wherein said recombinant adenovirus is Onyx 051.

26. (Objected To) The method of treating cancer of claim 13, wherein said recombinant adenovirus is Onyx 053.

27. (Objected To) The method of treating cancer of claim 11, wherein said mutated E1B-55K protein comprises a single amino acid substitution mutation in amino acid 240 or 260.

28. (Rejected) The method of treating cancer of claim 11, wherein replication of said recombinant adenovirus is cold insensitive.

29-32. (Canceled)

33. (Rejected) A method of treating a cancer, characterized by a tumor comprising neoplastic cells that substantially lack p53 function, in a patient in need of the treatment, comprising

administering chemotherapy to said patient,

administering by direct injection into the tumor a dose of a recombinant adenovirus, said recombinant adenovirus comprising a mutation in the E1B-55K gene, said gene encoding a mutated E1B-55K protein comprising a single amino acid substitution mutation, said single amino acid substitution mutation reducing the ability of said mutated E1B-55K protein to bind to the tumor suppressor p53 when compared to the ability of wild-type E1B-55K protein to bind to the tumor suppressor p53 and said recombinant adenovirus has the further property of retaining late viral function, and

allowing sufficient time for said recombinant adenovirus to infect neoplastic cells of said cancer.

34. (Canceled)

35. (Objected To) The method of claim 33, wherein said adenovirus is Onyx 051 or Onyx 053.

36. (Objected To) The method of treating cancer of claim 35, wherein said recombinant adenovirus is Onyx 051.

37. (Objected To) The method of treating cancer of claim 35, wherein said recombinant adenovirus is Onyx 053.

38. (Objected To) The method of treating cancer of claim 33, wherein said mutated E1B-55K protein comprises a single amino acid substitution mutation in amino acid 240 or 260.

39. (Rejected) The method of treating cancer of claim 33, wherein replication of said recombinant adenovirus is cold insensitive.

40. (Rejected) The method of treating cancer of claim 33, wherein said treatment is repeated.

41-47. (Canceled)

EVIDENCE APPENDIX

Appellants rely on the teachings of the specification as discussed herein above, as well as appellants' amendments to the claims and specification, including, the Response to Restriction Requirement and Amendment, dated 3 April 2006, the Response and Amendment, dated 19 September 2006, the Response to Final Rejection and Amendment, dated 16 April 2007, the Petition to the Director of Technology Center 1600 under 37 C.F.R. §1.181 Regarding an Improper Action by the Examiner in *Ex Parte* Prosecution, dated 9 July 2007, the Response to Non-Final Office Action, dated 9 October 2007, and the Response to Final Rejection, dated 15 July 2008.

Appellants also rely on "Replication-selective oncolytic adenoviruses: virotherapy aimed at genetic targets in cancer," by David Kirn, Oncogene **19**:6660-6669 (2000), cited by the Examiner in the Office action, dated 19 June 2006 (Evidence Appendix A, below).

Evidence Appendix A

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Oncogene (2000) 19, 6660–6669

www.nature.com/onc

Replication-selective oncolytic adenoviruses: virotherapy aimed at genetic targets in cancer

David Kirn*,[1]

[1]Imperial Cancer Research Fund, Program for Viral and Genetic Therapy of Cancer, Imperial College School of Medicine, Hammersmith Hospital, DuCane Road, London, W12 0NN, UK

Oncogene (2000) 19, 6660–6669.

Keywords: gene therapy; adenovirus; oncolytic; replication-selective

Introduction

The clinical utility of any cancer treatment is defined by both its antitumoral potency and its therapeutic index between cancerous and normal cells. Chemotherapy for metastatic solid tumors generally fails due to an insufficient therapeutic index and/or insufficient antitumoral potency. Although standard agents target a variety of different structures within cancer cells, almost all of them are thought to kill cancer cells through the induction of apoptosis. As a result, apoptosis-resistant clones develop following standard therapies, even if numerous high-dose chemotherapeutic agents are used in combination. Novel therapeutic approaches must therefore have not only greater potency and greater selectivity than currently available treatments, they should also have novel mechanisms of action that will not be subject to cross-resistance with existing approaches (i.e. efficacy should not be exclusively dependent on apoptosis induction in cancer cells).

Replication-selective oncolytic viruses (virotherapy) appear to have these characteristics. Viruses have evolved to infect cells, replicate, induce cell death, release of viral particles, and finally to spread in human tissues. Replication in tumor tissue leads to amplification of the input dose at the tumor site, while a lack of replication in normal tissues can result in efficient clearance and reduced toxicity (Figure 1). Selective replication within tumor tissue can theoretically increase the therapeutic index of these agents dramatically. In addition, viruses kill cells by a number of unique mechanisms. In addition to direct lysis at the conclusion of the replicative cycle, viruses can kill cells through expression of toxic proteins, induction of both inflammatory cytokines and T-cell-mediated immunity, and enhancement of cellular-sensitivity to their effects. Therefore, since activation of classical apoptosis pathways in the cancer cell is not the exclusive mode of killing, cross-resistance with standard chemotherapeutics or radiotherapy is much less likely to occur.

Revolutionary advances in molecular biology and genetics have led to a fundamental understanding of both (1) the replication and pathogenicity of viruses and (2) carcinogenesis. These advances have allowed novel agents to be engineered to enhance their safety and/or their antitumoral potency. Over the past decade, genetically-engineered viruses in development have included adenoviruses, herpesviruses and vaccinia. Viruses with inherent tumor-selectivity have been characterized and include reovirus, autonomous parvoviruses, Newcastle disease virus, measles virus strains and vesicular stomatitis virus (Kirn, 2000a). Each of these agents has shown tumor selectivity *in vitro* and/or *in vivo*, with many of these agents following intratumoral, intraperitoneal and/or intravenous routes of administration.

Although preclinical data reported with these agents has been encouraging, many critical questions have awaited results from clinical trials. Viral agents like adenovirus have complex biologies, potentially including species-specific interactions with host cell machinery and/or immune response effectors (Wold *et al.*, 1994; Sparer *et al.*, 1996). Antitumoral efficacy and safety studies with these viruses have been performed in rodent or primate models, and all published animal tumor model data with replication-selective adenoviruses has come from immunodeficient mouse-human tumor xenograft models (Rodriguez *et al.*, 1997; Heise *et al.*, 1999a,b). Therefore, data from cancer patients has been eagerly awaited. Now, after over 5 years of clinical research with *dl*1520, roughly 15 clinical trials have been completed and recently analysed involving approximately 250 patients.

This article will review the discovery and development of replication-selective oncolytic adenoviruses, with an emphasis on recently-acquired data from phase I and II clinical trials. The goal will be to summarize (1) the genetic targets and mechanisms of selectivity for these agents; (2) clinical trial data and what it has taught us to date about the promise but also the potential hurdles to be overcome with this approach; (3) future approaches to overcome these hurdles.

Attributes of replication-selective adenoviruses for cancer treatment

A number of efficacy, safety and manufacturing issues need to be assessed when considering a virus species for development as an oncolytic therapy (Kirn, 2000a). First, by definition the virus must replicate in and destroy human tumor cells. An understanding of the genes modulating infection, replication or pathogenesis is necessary for rational engineering of the virus. Since most solid human tumors have relatively low growth fractions, the virus should infect both cycling and non-cycling cells. In addition, receptors for viral entry must be expressed on the target tumor(s) in patients

*Correspondence: D Kirn

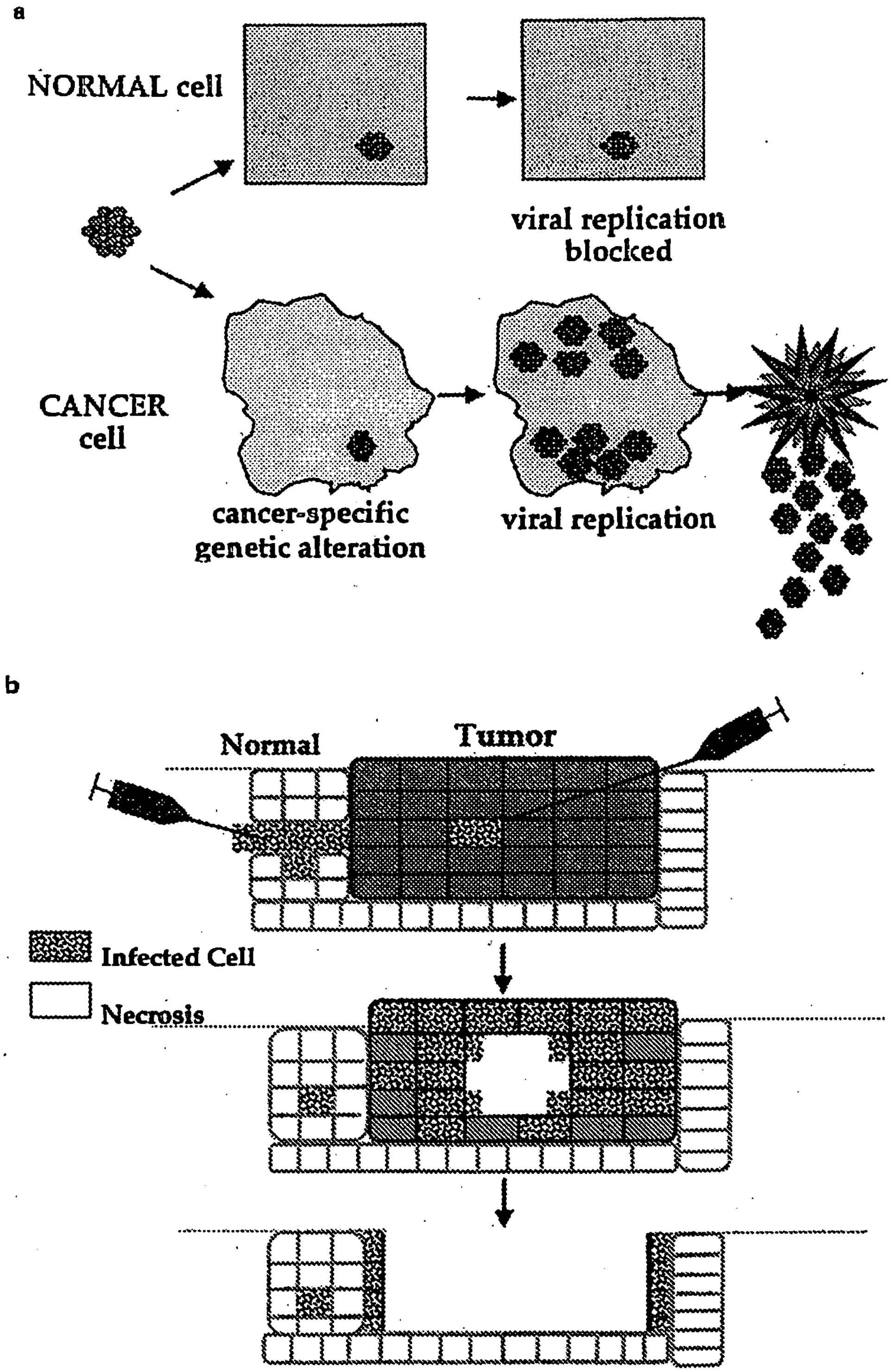

Figure 1 Schematic representation of tumor-selective viral replication and cell killing (a) and tumor-selective tissue necrosis (b)

(Wickham *et al.*, 1996). From a safety standpoint, the parental wildtype virus should ideally cause only mild, well-characterized human disease(s). Non-integrating viruses have potential safety advantages, as well. A genetically-stable virus is desirable from both safety and manufacturing standpoints. Finally, the virus must be amenable to high-titer production and purification under Good Manufacturing Practices (GMP) guidelines for clinical studies. Human adenoviruses have these characteristics and are therefore excellent oncolytic virus candidates (Heise and Kirn, 2000).

Biology of human adenovirus

Adenovirus biology is reviewed in detail elsewhere (Shenk, 1996). Roughly 50 different serotypes of human adenovirus have been discovered; the two most commonly studied are types 2 and 5 (group C). Adenoviruses have linear, double-stranded DNA genomes of approximately 38 kB. The capsid is non-enveloped and is comprised of the structural proteins hexon, fiber (binds coxsackie and adenovirus receptor-CAR), penton (binds $\alpha_v\beta_{3,5}$ integrins for virus inter-

nalization). The adenovirus life-cycle includes the following steps: (1) virus entry into the cell following CAR and integrin binding, (2) release from the endosome and subsequent entry into the nucleus, (3) expression of early region gene products, (4) cell entry into S phase, (5) prevention of p53-dependent and -independent apoptosis, (6) shut-off of host cell protein synthesis, (7) viral DNA replication, (8) viral structural protein synthesis, (9) virion assembly in the nucleus, (10) cell death and (11) virus release. The E3 region encodes a number of gene products responsible for immune response evasion (Wold *et al.*, 1995) (Dimitrov *et al.*, 1997). The gp19kD protein inhibits MHC-class I expression on the cell surface (i.e. avoidance of cytotoxic T-lymphocyte-mediated killing) (Hermiston *et al.*, 1993), and the E3 10.4/14.5 kD (RID complex) and 14.7 kD proteins inhibit apoptosis mediated by FasL or tumor necrosis factor (TNF) (Dimitrov *et al.*, 1997; Shisler *et al.*, 1996).

Table 1 Potential mechanisms of antitumoral efficacy with replication-selective adenoviruses

Mechanism	*Examples of adenoviral genes modulating effect*
I. Direct cytotoxicity due to viral proteins	E3 11.6 kD E4ORF4
II. Augmentation of antitumoral immunity	
CTL infiltration, killing	E3 gp19 kD*
Tumor cell death, antigen release	E3 11.6 kD
Immunostimulatory cytokine induction	E3 10.4/14.5, 14.7 kD*
Antitumoral cytokine induction (e.g. TNF)	E3 10.4/14.5, 14.7 kD*
Enhanced sensitivity to cytokines (e.g. TNF)	E1A
III. Sensitization to chemotherapy	Unknown (?E1A, others)
IV. Expression of exogenous therapeutic genes	n.a.

*Viral protein inhibits antitumoral mechanism. CTL, cytotoxic T-lymphocyte. TNF, tumor necrosis factor. NA, not applicable

Mechanisms of adenovirus-mediated cell killing

Adenovirus replication within a target tumor cell can lead to cell destruction by several mechanisms (Table 1). Viral proteins expressed late in the course of infection are directly cytotoxic, including the E3 11.6 kD adenovirus death protein (Tollefson *et al.*, 1996) and E4ORF4 [Branton, 1999 #1920]. Deletion of these gene products results in a significant delay in cell death. In addition, E1A expression early during the adenovirus life-cycle induces cell sensitivity to tumor necrosis factor (TNF)-mediated killing (Gooding, 1994). This effect is inhibited by the E3 proteins 10.4/14.5 and 14.7; deletion of these E3 proteins leads to an increase in TNF expression *in vivo* and enhanced cell sensitivity to TNF (Sparer *et al.*, 1996). Finally, viral replication in and lysis of tumor cells has been shown to promote the induction of cell-mediated immunity to uninfected tumor cells in model systems with other viruses (Toda *et al.*, 1999; Martuza, 2000); whether this will occur in patients and with adenovirus remains to be determined.

Approaches to optimizing tumor-selective adenovirus replication

Two broad approaches are currently being used to engineer tumor-selective adenovirus replication. One is to limit the expression of the E1A gene product to tumor tissues through the use of tumor- and/or tissue-specific promoters. E1A functions to stimulate S phase entry and to transactivate both viral and cellular genes that are critical for a productive viral infection (Whyte *et al.*, 1988). A second broad approach to optimizing tumor selectivity is to delete gene functions that are critical for efficient viral replication in normal cells but are expendable in tumor cells (described below).

Tissue- or tumor-specific promoters can replace endogenous viral sequences in order to restrict viral replication to a particular target tissue. For example, the prostate-specific antigen (PSA) promoter/enhancer element has been inserted upstream of the E1A gene; the result is that viral replication correlates with the level of PSA expression in a given cell (Rodriguez *et al.*, 1997). This virus, CN706 (Calydon Pharmaceuticals, CA, USA), is currently in a phase I clinical trial of intratumoral injection for patients with locally-recurrent prostate carcinoma. A second prostate-specific enhancer sequence has subsequently been inserted upstream of the E1B region (Yu *et al.*, 1999); the use of these two prostate-specific enhancer elements to drive separate early gene regions has led to improved selectivity over the first generation virus (Yu *et al.*, 1999). A similar approach has been pursued by other groups using tissue-specific promoters to drive E1A expression selectively in specific carcinomas (e.g. alpha-fetoprotein, carcinoembryonic antigen, MUC-1) (Hallenbeck, 1999; Kurihara *et al.*, 2000).

A second general approach is to complement loss-of-function mutations in cancers with loss-of-function mutations within the adenovirus genome. Many of the same critical regulatory proteins that are inactivated by viral gene products during adenovirus replication are also inactivated during carcinogenesis (Barker and Berk, 1987; Nielsch *et al.*, 1991; Sherr, 1996; Olson and Levine, 1994). Because of this convergence, the deletion of viral genes that inactivate these cellular regulatory proteins can be complemented by genetic inactivation of these proteins within cancer cells (Heise *et al.*, 1997b; Kirn *et al.*, 1998a). The deletion approach was first described by Martuza *et al.* (1991) with herpesviruses; the thymidine kinase gene (*dlsptk*) and subsequently the ribonucleotide reductase gene (G207) were deleted (Mineta *et al.*, 1995). Two adenovirus deletion mutation approaches have subsequently been described (see below).

E1A-CR2 region deletion mutants

Mutants in the E1A conserved region 2 (CR2) are defective in pRB binding (Whyte *et al.*, 1989; Hu *et al.*, 1990). These viruses are being evaluated for use against tumors with pRB pathway abnormalities (e.g. loss of the G1-S checkpoint) (Heise *et al.*, 2000a; Kirn *et al.*, 1998a; Fueyo *et al.*, 2000). The delta-24 E1A-CR2

mutant virus was able to efficiently replicate in tumor cell lines lacking functional pRB, while replication was significantly inhibited by reintroduction of wildtype RB protein into a tumor cell line lacking functional pRB; both *in vitro* and *in vivo* efficacy were demonstrated (Fueyo *et al.*, 2000). With *dl*922/947, a very similar E1A-CR2 mutant, S phase induction and viral replication are significantly inhibited in quiescent normal cells, whereas replication and cytopathic effects proceed efficiently in tumor cells; interestingly, *dl*922/947 demonstrates significantly greater potency than *dl*1520 both *in vitro* and *in vivo* (Kirn *et al.*, 1998a; Heise *et al.*, 2000a), and in a nude mouse-human tumor xenograft model, intravenously administered *dl*922/947 had significantly superior efficacy to even wildtype adenovirus (Heise *et al.*, 2000a). Unlike the complete deletion of E1B-55 kD in *dl*1520, these mutations in E1A are targeted to a single conserved region and may therefore leave intact other important functions of the gene product; therefore, viral potency is not attenuated.

E1B-55 kD gene deletion mutant: *dl*1520

*dl*1520 (Onyx-015) was the first adenovirus described to mirror the gene deletion approach pioneered by Martuza with herpesvirus. Bischoff *et al.* (1996) hypothesized that an adenovirus with deletion of a gene encoding a p53-binding protein, E1B-55 kD, would be selective for tumors that already had inhibited or lost p53 function. p53 function is lost in the majority of human cancers through mechanisms including gene mutation, overrexpression of p53-binding inhibitors (e.g. mdm2, human papillomavirus E6) and loss of the p53-inhibitory pathway modulated by p14^{ARF} (Scheffner *et al.*, 1991; Zhang *et al.*, 1998; Hollstein *et al.*, 1991). However, the precise role of p53 in the inhibition of adenoviral replication has not been defined to date. In addition, other adenoviral proteins also have direct or indirect effects on p53 function (e.g. E4orf6, E1B 19 kD, E1A) (Dobner *et al.*, 1996). Finally, E1B-55 kD itself has important viral functions that are unrelated to p53 inhibition (e.g. viral mRNA transport, host cell protein synthesis shut-off) (Yew *et al.*, 1994) (Figure 2).

Not surprisingly, therefore, the role of p53 in the replication-selectivity of *dl*1520 has been difficult to confirm despite extensive *in vitro* experimentation by many groups. E1B-55 kD gene deletion was associated with decreased replication and cytopathogenicity in p53(+) tumor cells versus matched p53(−) tumor cells, relative to wildtype adenovirus, in RKO and H1299 cells (Bischoff *et al.*, 1996; Harada and Berk, 1999; Rogulski *et al.*, 2000). However, conflicting data on the role of p53 in modulating *dl*1520 replication and/or c.p.e. has come from different cell systems; no p53 effect was demonstrated in matched U2OS cells, for example (Rothmann *et al.*, 1998). It is clear that many other cellular factors independent of p53 play critical roles in determining the sensitivity of cells to *dl*1520 (Ries *et al.*, 2000; Harada and Berk, 1999; Heise *et al.*, 1997a; Goodrum and Ornelles, 1997, 1998). Clinical trials were ultimately necessary to determine the selectivity and clinical utility of *dl*1520 (see below).

Clinical trial results with replication-competent adenoviruses in cancer patients

Clinical trial results with wild-type adenovirus: flawed study design

Over the last century a diverse array of viruses were injected into cancer patients by various routes, including adenovirus, Bunyamwara, coxsackie, dengue, feline panleukemia, Ilheus, mumps, Newcastle Disease virus, vaccinia and West Nile (Kirn, 2000a; Southam and Moore, 1952; Asada, 1974; Smith *et al.*, 1956). These studies illustrated both the promise and the hurdles to overcome with oncolytic viral therapy. Unfortunately, these previous clinical studies were not performed to current clinical research standards, and therefore none give interpretable and definitive results. At best, these studies are useful in generating hypotheses that can be tested in future trials.

Although suffering from many of the trial design flaws listed below, a trial with wild-type adenovirus is one of the most useful for hypothesis generation but also for illustrating how clinical trial design flaws severely curtail the utility of the study results. The knowledge that adenoviruses could eradicate a variety of tumor cells *in vitro* led to a clinical trial in the 1950's with wild-type adenovirus. Ten different serotypes were used to treat 30 cervical cancer patients (Smith *et al.*, 1956). Forty total treatments were administered by either direct intratumoral injection ($n=23$), injection into the artery perfusing the tumor ($n=10$), treatment by both routes ($n=6$) or intravenous administration ($n=1$). Characterization of the material injected into patients was minimal. The volume of viral supernatant injected is reported, but actual viral titers/doses are not; injection volumes (and by extension doses) varied greatly. When possible, the patients were treated with a serotype to which they had no neutralizing antibodies present. Corticosteroids were administered as non-specific immunosuppressive agents in roughly half of the cases. Therefore, no two patients were treated in identical fashion.

Nevertheless, the results are intriguing. No significant local or systemic toxicity was reported. This relative safety is notable given the lack of pre-existing immunity to the serotype used and concomitant corticosteroid use in many patients. Some patients reported a relatively mild viral syndrome lasting 2–7 days (severity not defined); this viral syndrome resolved spontaneously. Infectious adenovirus was recovered from the tumor in two-thirds of the patients for up to 17 days post-inoculation.

Two-thirds of the patients had a 'marked to moderate local tumor response' with necrosis and ulceration of the tumor (definition of 'response' not reported). None of the seven control patients treated with either virus-free tissue culture fluid or heat-inactivated virus had a local tumor response (statistical significance not reported). Therefore, clinically evident tumor necrosis was only reported with viable virus. Neutralizing antibodies increased within 7 days after administration. Although the clinical benefit to these patients is unclear, and all patients eventually had tumor progression and died, this study did demonstrate that wildtype adenoviruses can be safely administered to patients and that these viruses can

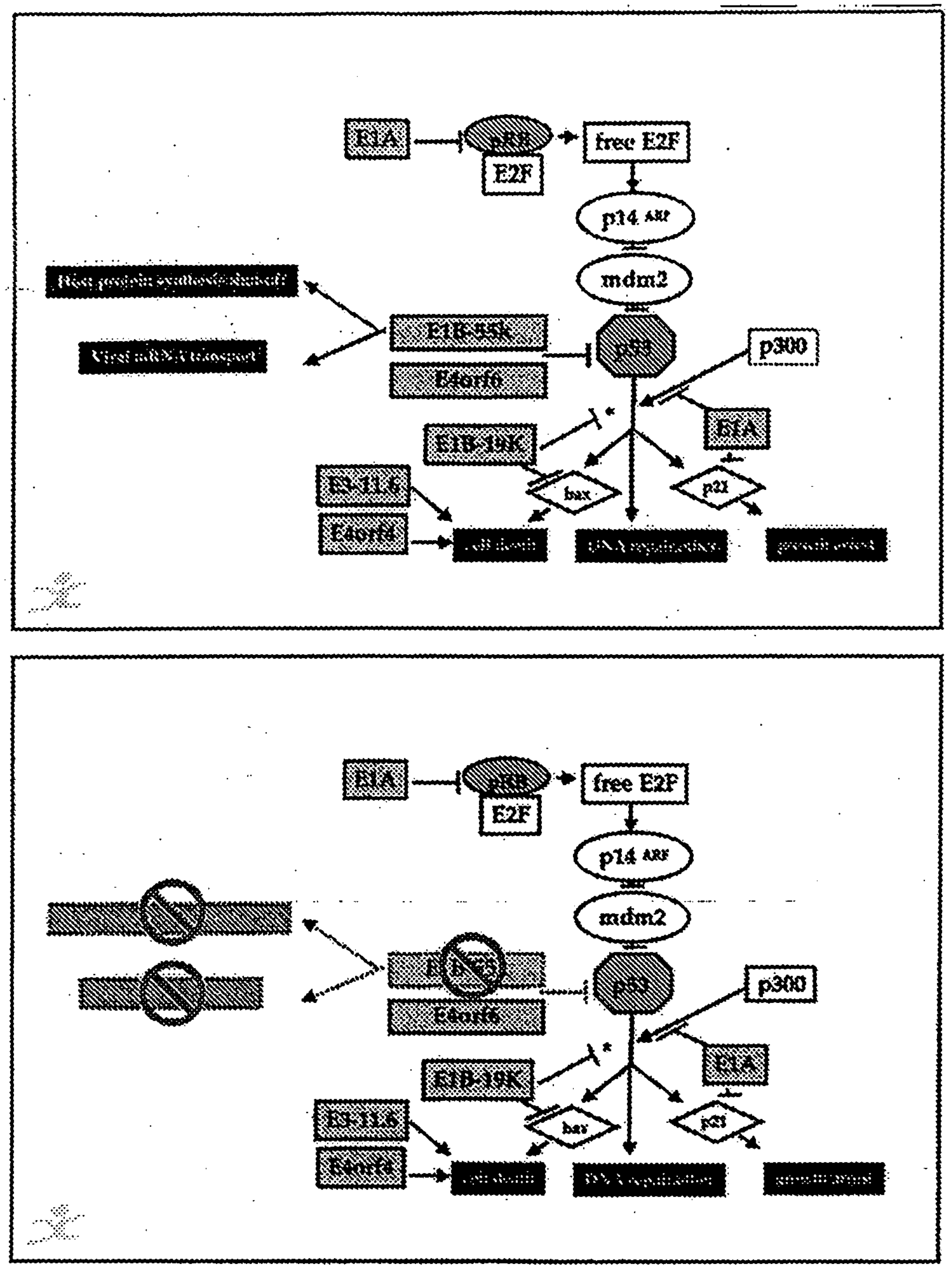

Figure 2 Diagram of both p53 pathway interactions with adenoviral gene products and functions of E1B-55 kD: complexity of cancer cell and adenoviral biology. (a) Note that adenoviral proteins (light gray) target multiple components of this pathway at sites upstream of p53, downstream of p53 and at the level of p53 itself. Examples of p53-regulated cell functions are shown (black boxes). In addition, the known functions of E1B-55 kD are shown (dark gray). (b) Graphic representation of the consequences of E1B-55 kD gene deletion. In addition to the loss of p53 binding when E1B-55 kD was deleted in *dl*1520 (Onyx-015), other important viral functions are also lost

replicate and cause necrosis in solid tumors despite a humoral immune response. The maximally-tolerated dose, dose-limiting toxicity, objective response rate and time to tumor progression, however, remain unknown for any of these serotypes by any route of administration.

A novel staged approach to clinical research with replication-selective viruses: the example of dl*1520 (Onyx-015)*

For the first time since viruses were first conceived as agents to treat cancer over a century ago, we now have definitive data from numerous phase I and II clinical trials with a well-characterized and well-quantitated virus. *dl*1520 (Onyx-015, now CI-1042, Pfizer, Inc.) is a novel agent with a novel mechanism of action. This virus was to become the first virus to be used in humans that had been genetically-engineered for replication-selectivity.

We predicted that both toxicity and efficacy would be dependent on multiple factors including (1) the inherent ability of a given tumor to replicate and shed the virus, (2) the location of the tumor to be treated (e.g. intracranial vs peripheral) and (3) the route of administration of the virus. In addition, we felt it would be critical to obtain biological data on viral replication, antiviral immune responses and their

relationship to antitumoral efficacy in the earliest phases of clinical research.

We therefore designed and implemented a novel staged clinical research and development approach with this virus (Figure 3). The goal of this approach was to sequentially increase systemic exposure to the virus only after safety with more localized delivery had been demonstrated. Following demonstration of safety and biological activity by the intratumoral route, trials were sequentially initiated to study intracavitary instillation (initially intraperitoneal), intra-arterial infusion (initially hepatic artery) and eventually intravenous administration. In addition, only patients with advanced and incurable cancers were initially enrolled on trials. Only after safety had been demonstrated in terminal cancer patients were trials initiated for patients with premalignant conditions. Finally, clinical trials of combinations with chemotherapy were initiated only after the safety of *dl*1520 as a single agent had been documented by the relevant route of administration.

Results from clinical trials with *dl*1520 (Onyx-015, or CI-1042)

Toxicity

No maximally-tolerated dose or dose-limiting toxicities were identified at doses up to 2×10^{12} particles administered by intratumoral injection. Flu-like symptoms and injection-site pain were the most common associated toxicities (Ganly *et al.*, 2000). This safety is remarkable given the daily or even twice-daily dosing that was repeated every 1–3 weeks in the head and neck region or pancreas (Nemunaitis *et al.*, 2000b).

Intraperitoneal, intra-arterial and intravenous administration were also remarkably well-tolerated, in general. Intraperitoneal administration was feasible at doses up to 10^{13} particles divided over five days (Vasey *et al.*, 2000). The most common toxicities included fever, abdominal pain, nausea/vomiting and bowel motility changes (diarrhea, constipation). The severity of the symptoms appeared to correlate with tumor burden. Patients with heavy tumor burdens reached a maximally-tolerated dose at 10^{12} particles (dose-limiting toxicities were abdominal pain and diarrhea), whereas patients with a low tumor burden tolerated 10^{13} without significant toxicity.

No dose-limiting toxicities were reported following repeated intravascular injection at doses up to 2×10^{12} particles (hepatic artery) (Reid *et al.*, 2000) or 2×10^{13} particles (intravenous) (Nemunaitis *et al.*, 2000a). Fever, chills and asthenia following intravascular injection were more common and more severe than after intratumoral injections (grade 2–3 fever and chills vs grade 1). Dose-related transaminitis was reported infrequently. The transaminitis was typically transient (<10 days) and low-grade (grade 1–2) and was not clinically-relevant. Further dose escalation was limited by supply of the virus.

Viral replication

Viral replication has been documented at early time-points after intratumoral injection in head and neck cancer patients (Figure 4) (Nemunaitis *et al.*, 2000b,c). Roughly 70% of patients had evidence of replication on days 1–3 after their last treatment. In contrast, day 14–17 samples were uniformly negative. Intratumoral injection of liver metastases (primarily colorectal) led to similar results at the highest doses of a phase I trial. Patients with injected pancreatic tumors, in contrast, showed no evidence of viral replication by plasma PCR or fine needle aspiration. Similarly, intraperitoneal *dl*1520 could not be shown to reproducibly infect ovarian carcinoma cells within the peritoneum. Therefore, different tumor types can vary dramatically in their permissiveness for viral infection and replication.

Proof-of-concept for tumor infection following intra-arterial (Reid *et al.*, 2000) or intravenous (Nemunaitis *et al.*, 2000a) administration with human adenovirus has also been achieved. Approximately half of the roughly 25 patients receiving hepatic artery infusions of 2×10^{12} particles were positive by PCR 3-5 following treatment. Patients with elevated neutralizing antibody titers prior to treatment were substantially less likely to have evidence of viral replication 3–5 days post-treatment. Three of four patients with metastatic carcinoma to the lung treated intravenously with $\geqslant 2 \times 10^{12}$ particles were positive for replication by PCR on day 3 (± 1). Therefore, it is feasible to infect distant tumor nodules following intravenous or intra-arterial administration.

Immune response

Neutralizing antibody titers to the coat (Ad5) of dl1520 were positive but relatively low in roughly 50–60% of all clinical trial patients at baseline (Nemunaitis *et al.*, 2000b). Antibody titers increased uniformly following administration of *dl*1520 by any of the routes tested, in some cases to levels >1:80 000. Antibody increases occurred regardless of evidence for replication or shedding into the bloodstream (Nemunaitis *et al.*, 2000b). Acute inflammatory cytokine levels were determined prior to treatment (by hepatic artery infusion), 3 h post- and 18 h post-treatment: IL-1, IL-6, IL-10, interferon-gamma, tumor necrosis factor. Significant increases were demonstrated within 3 h for IL-1, IL-6, tumor necrosis factor and to a lesser extent interferon-gamma; all cytokines were back down to pretreatment levels by 18 h (Reid *et al.*, 2000). In contrast, IL-10 did not increase until 18 h.

Efficacy with dl*1520 (Onyx-015) as a single agent*

Two Phase II trials enrolled a total of 40 patients with recurrent head and neck cancer (Nemunaitis *et al.*, 2000b,c). Tumors were treated very aggressively with 6–8 daily needle passes for 5 consecutive days (30–40 needle passes per 5 day cycle; $n=30$) and 10–15 per day on a second trial (50–75 needle passes per cycle; $n=10$). The median tumor volume on these studies was approximately 25 cm^3; an average cm^3 of tumor therefore received an estimated 4–5 needle passes per cycle. Despite the intensity of this local treatment, the unconfirmed response rate at the injected site was only 14% and the confirmed local response rate was <10%. Interestingly, there was no correlation between evidence of antitumoral activity and neutralizing antibody levels at baseline or post-treatment (Nemunaitis *et al.*,

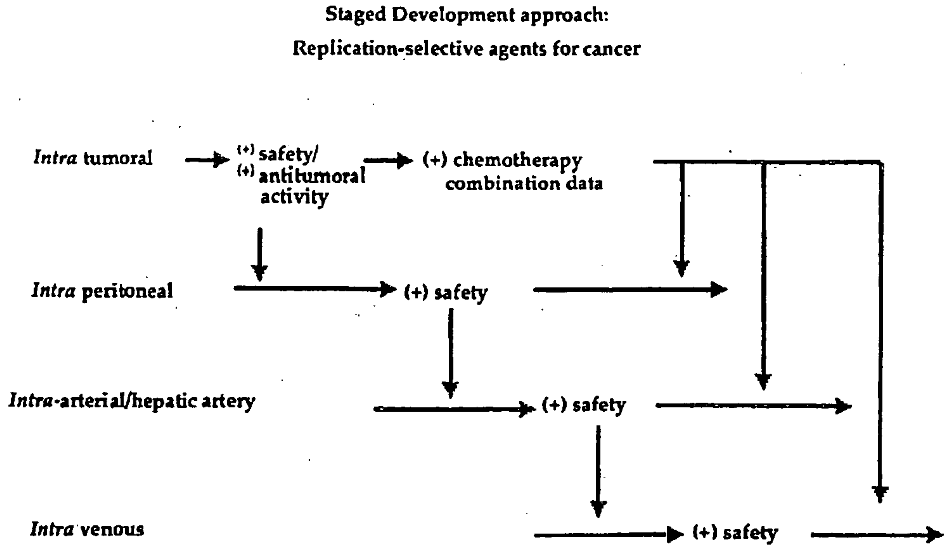

Figure 3 A staged clinical research and development approach for a replication-selective agent in cancer patients. Following demonstration of safety and biological activity by the intratumoral route, trials were sequentially initiated to study intracavitary instillation (initially intraperitoneal), intra-arterial infusion (initially hepatic artery) and eventually intravenous administration. In addition, only patients with advanced and incurable cancers were initially enrolled on trials. Only after safety had been demonstrated in terminal cancer patients were trials initiated for patients with premalignant conditions. Finally, clinical trials of combinations with chemotherapy were initiated only after the safety of *dl*1520 as a single agent had been documented by the relevant route of administration

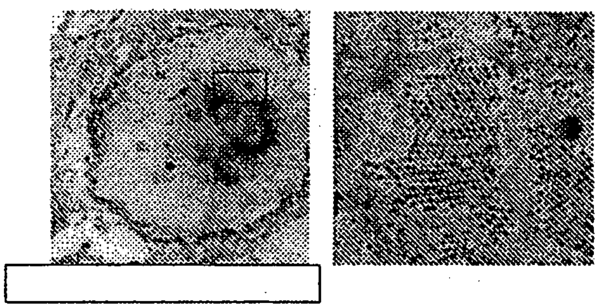

Figure 4 Replication of adenoviral agent (*dl*1520) in nucleus of squamous carcinoma cell in the head and neck region of a patient 3 days after intratumoral virus injection. The dark staining individual particles (arrow) and clusters within the nucleus (box) are adenoviral particles

2000b). No objective responses were demonstrated in patients with tumor types that could not be so aggressively injected (due to their deep locations), although some evidence of minor shrinkage or necrosis was obtained. In summary, single agent responses across all studies were rare, and therefore combinations with chemotherapy were explored.

Efficacy in combination with chemotherapy: potential synergy discovered

Evidence for a potentially-synergistic interaction between adenoviral therapy and chemotherapy has been obtained on multiple trials. Encouraging clinical data has been obtained in patients with recurrent head and neck cancer treated with intratumoral *dl*1520 in combination with intravenous cisplatin and 5-fluorouracil (Khuri *et al.*, 2000). Thirty-seven patients were treated and 19 responded (54%, intent-to-treat; 63%, evaluable); this compares favorably with response rates to chemotherapy alone in previous trials (30–40%, generally). The time-to-tumor progression was also superior to previously reported studies. However, comparisons to historical controls are unreliable. We therefore used patients as their own controls whenever possible ($n=11$ patients). Patients with more than one tumor mass had a single tumor injected with *dl*1520 while the other mass(es) was left uninjected. Since both masses were exposed to chemotherapy, the effect of the addition of viral therapy to chemotherapy could be assessed. The *dl*1520-injected tumors were significantly more likely to respond ($P=0.017$) and less likely to progress ($P=0.06$) than were non-injected tumors. Non-injected control tumors that progressed on chemotherapy alone were subsequently treated with Onyx-015 in some cases; two of the four injected tumors underwent complete regressions. This data illustrates the potential of viral and chemotherapy combinations. The clinical utility of *dl*1520 in this indication will be definitively determined in a phase III randomized trial.

A phase I/II trial of *dl*1520 administered by hepatic artery infusion in combination with intravenous 5-fluorouracil and leukovorin was carried out ($n=33$ total) (Reid *et al.*, 2000). Following phase I dose escalation, 15 patients with colorectal carcinoma who had previously failed the same chemotherapy were treated with combination therapy after failing to respond to *dl*1520 alone; one patient underwent a partial response and 10 had stable disease (2–7+ months). Chemosensitization of colorectal liver metastases is therefore possible via hepatic artery infusions, although the magnitude and frequency of this effect remains to be determined. In contrast, data from a phase I/II trial studying the combination of *dl*1520 and gemcitabine chemotherapy were disappointing ($n=21$); the combination resulted in only two responses, and these patients had not received prior gemcitabine (Hecht *et al.*, 2000). Therefore, potential synergy was demonstrated with *dl*1520 and chemotherapy in two tumor types that supported viral replication (head and

neck, colorectal). but not in a tumor type that was resistant to viral replication (pancreatic).

Results from clinical trials with *dl*1520 (Onyx-015): Summary

*dl*1520 has been well-tolerated at the highest practical doses that could be administered ($2\times10^{12}-2\times10^{13}$ particles) by intratumoral, intraperitoneal, intra-arterial and intravenous routes. The lack of clinically-significant toxicity in the liver or other organs was notable. Flu-like symptoms (fever. rigors, asthenia) were the most common toxicities and were increased in patients receiving intravascular treatment. Acute inflammatory cytokines (especially IL-1 and IL-6) increased within 3 h following intra-arterial infusion. Neutralizing antibodies increased in all patients, regardless of dose, route or tumor type. Viral replication was documented in head and neck and colorectal tumors following intratumoral or intra-arterial administration. Neutralizing antibodies did not block antitumoral activity in head and neck cancer trials of intratumoral injection. However, viral replication/shedding into the blood was inhibited by neutralizing antibodies; intra-arterial virus was more sensitive to antibody inhibition than was intratumorally injected virus. Single agent antitumoral activity was minimal ($\cong$15%) in head and neck cancers that could be repeatedly and aggressively injected. No objective responses were documented with single agent therapy in phase I or I/II trials in patients with pancreatic, colorectal or ovarian carcinomas. A favorable and potentially synergistic interaction with chemotherapy was discovered in some tumor types and by different routes of administration.

Future directions: Why has *dl*1520 (Onyx-015) failed to date as a single agent for refractory solid tumors?

Future improvements with this approach will be possible if the reasons for *dl*1520 failure as a single agent, and success in combination with chemotherapy, are uncovered. Factors that are specific to this adenoviral mutant, as well as factors that may be generalizable to other viruses, should be considered. Regarding this particular adenoviral mutant, it is important to remember that this virus is attenuated relative to wildtype adenovirus in most tumor cell lines *in vitro* and *in vivo*, including even p53 mutant tumors (Harada and Berk, 1999; Goodrum and Ornelles, 1997; Kirn *et al.*, 1998b; Rothmann *et al.*, 1998; Heise *et al.*, 2000a). This is not an unexpected phenotype since this virus has lost critical E1B-55 kD functions that are unrelated to p53, including viral mRNA transport. This attenuated potency is not apparent with other adenovirus mutants such as *dl*922/947 (Heise *et al.*, 2000a).

In addition, a second deletion in the E3 gene region (10.4/14.5 complex) may make this virus more sensitive to the antiviral effects of tumor necrosis factor; an immunocompetent animal model will need to be identified in order to resolve this issue. Factors likely to be an issue with any virus include barriers to intratumoral spread, antiviral immune responses and inadequate viral receptor expression (e.g. CAR, integrins). Viral coat modifications may be beneficial if inadequate CAR expression plays a role in the resistance of particular tumor types (Roelvink *et al.*, 1999; Douglas *et al.*, 1996).

Future directions: improving the efficacy of replication-selective agents

Mutations in the adenoviral genome can enhance selectivity and/or potency. For example, a promising adenoviral E1A CR-2 mutant (*dl*922/947) has been described that demonstrates not only tumor-selectivity (based on the G1-S checkpoint status of the cell) but also significantly greater antitumoral efficacy *in vivo* compared to *dl*1520 (all models tested) and even wildtype adenovirus (in a breast cancer metastasis model) (Kirn *et al.*, 1998a). Another very similar E1A mutant adenovirus has demonstrated replication and cytopathic effects based on the pRB status of the target cell (Fueyo *et al.*, 2000). Deletion of the E1B-19 kD gene (antiapoptotic bcl-2 homologue) is known to result in a 'large plaque' phenotype due to enhanced speed of cell killing (Chinnadurai, 1983). This observation has now been extended to multiple tumor cell lines and primary tumor cell cultures (Sauthoff *et al.*, 2000; Medina *et al.*, 1999). A similar phenotype resulted from overexpression of the E3-11.6 adenovirus death protein (Doronin *et al.*, 2000). It remains to be seen whether these *in vitro* observations are followed by evidence for improved efficacy *in vivo* over wildtype adenovirus.

Potency can also be improved by arming viruses with therapeutic genes (e.g. prodrug-activating enzymes and cytokines) (Hermiston, 2000; Hawkins *et al.*, 1999; Freytag *et al.*, 1998; Wildner *et al.*, 1999). Viral coat modifications may be beneficial if inadequate CAR expression plays a role in the resistance of particular tumor types (Roelvink *et al.*, 1999; Douglas *et al.*, 1996). Improved systemic delivery may require novel formulations or coat modifications, as well as suppression of the humoral immune response. Determination of the viral genes (e.g. E3-region) and immune response parameters mediating efficacy and toxicity will lead to immunomodulatory strategies. Finally, identification of the mechanisms leading to the potential synergy between replicating adenoviral therapy and chemotherapy may allow augmentation of this interaction (Heise *et al.*, 2000b). This understanding may then allow us to bolster this interaction.

Summary

Replication-selective oncolytic adenoviruses represent a novel cancer treatment platform. Clinical studies have demonstrated the safety and feasibility of the approach, including the delivery of adenovirus to tumors through the bloodstream (Heise *et al.*, 1999b; Reid *et al.*, 1999; Nemunaitis *et al.*, 1999). The inherent ability of replication-competent adenoviruses to sensitize tumor cells to chemotherapy was a novel discovery that has led to chemosensitization strategies. These data will support the further development of adenoviral agents, including second-generation constructs containing exogenous therapeutic genes to enhance

both local and systemic antitumoral activity (Heise and Kirn, 2000; Hermiston, 2000; Agha-Mohammadi and Lotze, 2000). In addition to adenovirus, other viral species are being developed including herpesvirus, vaccinia, reovirus and measles virus (Kirn, 2000a; Martuza, 2000; Norman and Lee, 2000; Mastrangelo *et al.*, 2000; Coffey *et al.*, 1998; Martuza *et al.*, 1991; Kirn, 2000b; Lattime *et al.*, 1996). Since intratumoral spread also appears to be a substantial hurdle for viral agents, inherently motile agents such as bacteria may hold great promise for this field (Low *et al.*, 1999; Sznol *et al.*, 2000).

Given the unknown predictive value of *in vitro* cell-based assays and murine tumor model systems for the efficacy and therapeutic index of replication-selective oncolytic adenoviruses in patients, we believe that encouraging adenoviral agents must be tested in well-designed clinical trials as soon as possible. Only then can the true therapeutic potential of these agents be realized.

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RELATED PROCEEDINGS APPENDIX

None.